THE ARMY'S UNIVERSITY AFFILIATED RESEARCH CENTER CHEMICAL/BIOLOGICAL COUNTERMEASURES

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In 2000, a National Biological and Chemical Countermeasures Program was created involving three major institutions: The University of Texas (UT), The University of South Florida, and Texas Tech University. This report considers the structure and technical achievements of The University of Texas University Affiliated Research Center (UARC) component and describes research activities from four UT campuses (Austin, Galveston Medical Branch, San Antonio and South West Medical School Dallas), the Texas Department of Health, the 6th Civil Support Team of the Texas National Guard, Austin Office of Emergency Management, and the Institute for Defense Analyses. The major research efforts are in sensors for threat agents, biomedical responses, and communication. During the first year rapid progress was achieved in several areas, including: the production of high affinity antibodies against anthrax toxin and brucella presented on external surfaces of bacteria; the production of antibodies against multiple pathogenic components; and identification of aptamers that bind ricin and recognize nuclear factors involved in inflammatory responses to threat agents. Intelligent software agents are being developed for belief maintenance and resource allocation. The integration of this effort with the Texas State Health Alert Network (HAN) and archival health data sets initiates our efforts in fusing countermeasures with biosurveillance responses.

INTRODUCTION

In the event of an actual CB attack, the components needed for a successful response will include: human and material resources needed to validate that an incident has occurred (e.g., sensor systems); physical and medical countermeasures (technologies for the rapid diagnosis and treatment of exposed targets or environments); novel counter-agents (antidotes, vaccines) that will be able to defeat the validated biological or chemical agent; health care and triage related to the treatment of people in the area of the affected target; effective communication; and an effective system that integrates these requirements. A team with expertise in all of these areas has been assembled which includes scientific components at the University of Texas. This integrated team is designed to assist the Department of Defense and relevant government agencies in comprehensively addressing the challenges ensuing from a biological/chemical incident. This team has expertise in antibody, genome, and aptamer based sensors, in rapid scale-up vaccines, the effect of B agents on experimental animals, hierarchical computer based communications, clinical diagnostics, and public health delivery response. The unique contributions of this program are the presence of leading edge research teams coupled with application teams that will transition discoveries to meet military needs and requirements.

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The Objective of the UARC Biological/Chemical Countermeasures Program is to disencumber the US Army Objective Force from operational and combat capability constraints posed by these threats. Three elements of this effort are sensor development, medical countermeasures and communications.

DISCUSSION

Our research team has been developing high affinity binders of threat agents and exploring platforms upon which the binders can be placed effectively. The high affinity binders include antibodies with $K_d<10^{-10}$, polynucleotide aptamers and cDNA probes. The laboratory of George Georgiou and Brent Iverson has developed a technology by which randomized portions of the variable region of immunoglobulins are expressed on the external surface of bacteria. The bacteria producing highest affinity antibody fragments against anthrax toxin and brucella are selected using fluorescent tagged antigen and a fluorescent activated cell sorter. The cells with the highest affinity binders were then grown to produce 10^7 cells that could bind antigen (Figure 1).

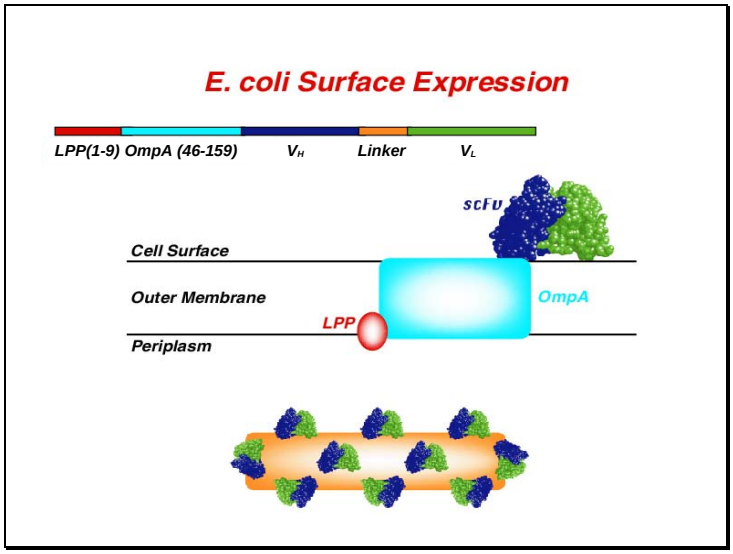
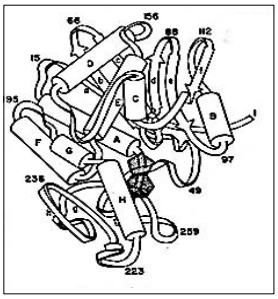
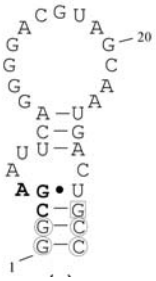


Figure 1. Expression of variable region of antibodies on E.coli surface.

The advantage of this technique is that the highest affinity antibodies against threat agents may be rapidly assessed for utility in field hardened sensor systems. The bacterial systems are stable and can be grown to requirements in a short period of time.

A different binding system has been explored by researchers in the laboratory of Andrew Ellington using polynucleotide aptamers (approximately 31 nucelotides in length) that have good affinity for the toxin ricin. The aptamers are low molecular weight materials that are synthesized in the laboratory and may be attached to various matrices used in sensor systems. In addition to serving as sensor elements, such compounds may have therapeutic value if the bound toxin is rendered inactive in the host. Figures 2a and 2b illustrate the technology.





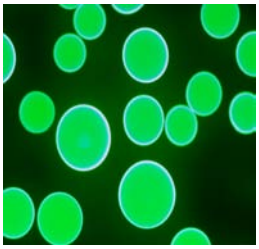
Anti-ricin aptamer

Aptamers against biological warfare targets

Schedule:

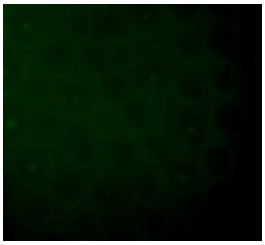
FY01: Aptamer against pathogenicity island
Aptamer against B. anthracis
Antibody against Botulinum toxin A

Figure 2a. Polynucleotide aptamers to ricin.



Positive

1. Avidin coupled agarose
2. Biotinylated-DNA
3. Labelled DNA complement



Negative

1. Avidin coupled agarose
2. Labelled DNA complement

Figure 2b. Visualization of aptamer target complexes.

The laboratory of Robert Shope is developing polynucleotide aptamers that bind transcription nuclear factors in macrophages (NFkB) involved in the initiation of inflammatory response following infection with a variety of biological agents including Pichinde virus. The nuclear factors are activated when stimulated by virulent agent but not avirulent material (Figure 3). The resulting thioaptamers are anticipated to have utility in the design of biologicals that can prevent massive inflammatory response and thereby protect forces from toxic shock resulting from threat agents.

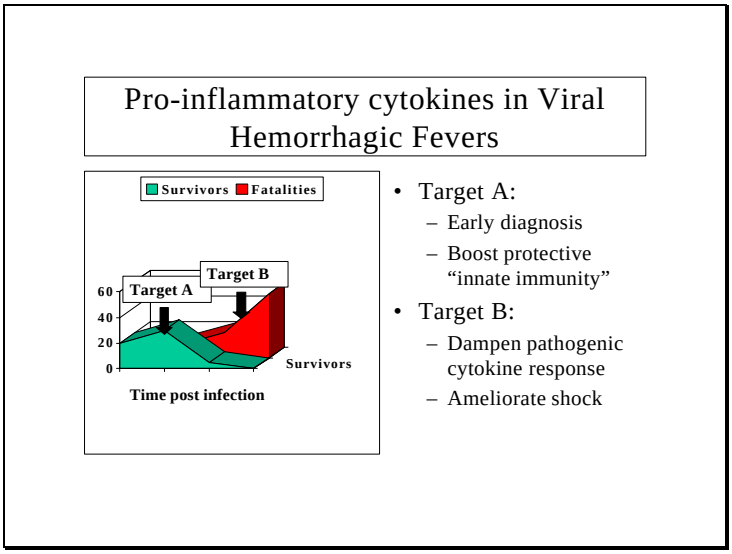


Figure 3. Aptamers for therapeutics against pro-inflammatory cytokines.

A fourth example of the progress made in sensors by the UARC is represented by the successes of Shelley Payne and colleagues. The genetic determinants that lead to pathogenicity of bacteria are factors that increase adhesion of bacteria to target tissues or increase transport of nutrients essential for the organism to grow in particular environments. These virulence factors are present in pathogenic shigella and E Coli ColV (Figure 4). The Payne group has sequenced the virulence factor aerobactin genes responsible for transport of heme iron.

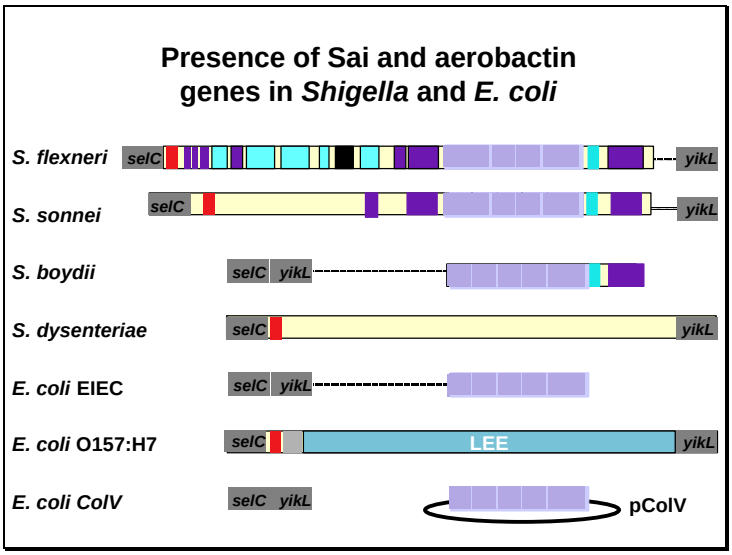


Figure 4. Virulence factors in Shigella and E. coli.

Three platforms for sensors are currently in exploration. One is designed by the team of John McDevitt and Eric Anslyn and is called the electronic tongue; a second utilizes a redox system that will detect polynucleotide complexes in a gel matrix designed by the group led by Adam Heller; and the third is an apatmer based sensor developed by James Chambers of UT San Antonio. The Chambers design has

utilized an anti-ricin aptamer constructed by Andrew Ellington. The McDevitt electronic tongue has shown utility in detecting an antigen-antibody complex.

An intelligent software system is being designed by K. Suzanne Barber. This has the attributes of addressing belief maintenance and resource allocation in a manner similar to that of the Encompass Program created by DARPA. A critical component of the Countermeasures Effort is to evaluate the credibility of incoming information and create a network that will enable distribution of the information. An illustration of this component is shown in Figure 5.

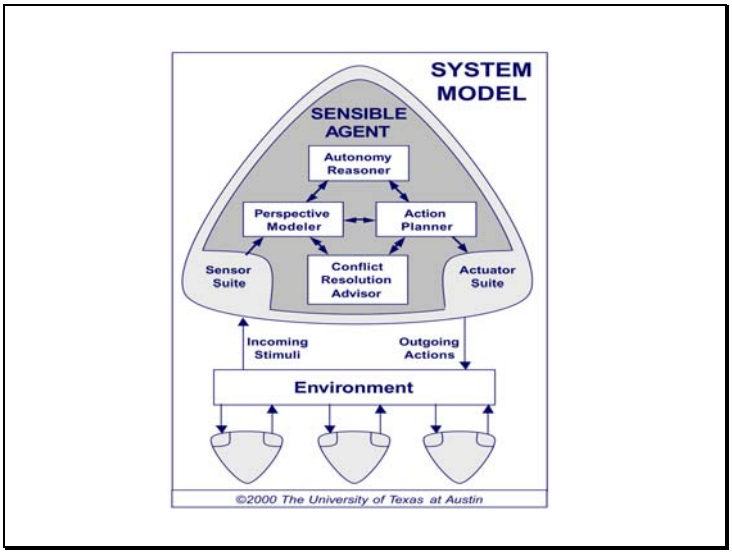


Figure 5. Intelligent software agents.

This communication system, as it develops, interfaces the sensors programs with the Texas Department of Health. The 6th Civil Support Team can provide secure means for transmission of critical information.

During the first year of funding the program has experienced rapid progress in all areas (Table 1).

TABLE 1. UARC accomplishments in first year.

Sensors	• Generated high affinity Ab's-anthracis toxin • Designed aptamer bases MEMS chip • Developed anti-ricin aptamers for detector elements • Detect 10^8 copies/microliter of 70 base DNA in 20 min; S/N ratio 7 • Characterize heme transport gene in pathogenicity island
Bio-Med	• Demonstrated new vaccine against multiple pathogens • Identified potential signatures for early alert disease outbreak • Developed compounds with potential protective properties against Pichinde virus
Communication	• Defined B/C Incident constituencies and their unique communications requirements • Identified "signature" flags in health system for early warning of B/C event • Identified ER facility and faculty at Hermann Hospital, Houston, with leading edge electronic data entry for surveillance

CONCLUSIONS

This integrated team is designed to assist the Department of Defense and other US government agencies in comprehensively addressing the challenges ensuing from a chemical/biological incident. This team has recognized expertise in antibody, genome, and aptamer based sensors, in rapid scale-up of vaccines, in knowledge of the effect of B agents on experimental animals, in hierarchical computer based communications and telemedicine, in clinical diagnostics, and in public health delivery response. The unique contributions of this program are the presence of leading edge research teams coupled with application teams that will transition discoveries to meet military needs and requirements.

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